Workflow protocol for the digitization of ethanol-preserved isopods to accelerate the process of species description

Justine L. Uebeler[1]*, Carla M. A. Rumpf[1]*, Valentin de Mazancourt[1] & Kristina von Rintelen[1]

(*Both authors contributed equally to this publication)

[1] Museum für Naturkunde – Leibniz Institute for Evolution and Biodiversity Science, Center for Integrative Biodiversity Discovery, Invalidenstraße 43, D-10115 Berlin, Deutschland

Corresponding author: Kristina von Rintelen (kristina.vonrintelen@mfn.berlin)

Abstract

This student project established and tested a digitization workflow with ethanol-preserved museum specimens at the Museum für Naturkunde (MfN) Berlin. Recently collected small isopod crustaceans (freshwater and terrestrial from Southeast Asia) were chosen as test organisms. This study aimed i) to test a digitization workflow for a possibly faster description and documentation of morphological characters for species descriptions, ii) to do a small test run for the digitization of ethanol-preserved museum specimens (wet collection), and iii) to test the use of museum internal Uniform Resource Identifiers (MfN URIs or NURIs) and QR code labels to easily connect a physical research object with a database.

The result was a three-step workflow protocol including 1) digitizing specimen-associated data, 2) digital imaging of specimens, and 3) photo editing and digital drawing. It produced high-quality illustrations ready to be used for scientific publications and species descriptions. The workflow itself proved to be efficient and time-saving but only provided that the entire equipment and software is fully functioning. It may not be suitable for the mass-digitization of wet collections in natural history museums but can help standardize and digitize the morphological part of a taxonomic species description. Furthermore, it can help exchange high-resolution digital images and digital drawings of habitus and taxonomic details of ethanol-preserved museum specimens below 5 cm with other taxonomists or digital images with a broader public.

Keywords

Crustacea; wet collection; taxonomy; morphology; technical workflow; digitization

Contents

1. Introduction

The term biodiversity refers to the variety of living organisms, the term digitization implies the use of digital technologies. Both are frequent buzzwords used in all possible contexts (in politics, bioeconomy, science, media, etc.) and are currently combined in various digitization projects of museum specimens and associated data in natural history collections and for various biodiversity research projects (e.g., Contreras, 2018; Hedrick et al., 2020; Nelson & Ellis, 2018).

Most digitization projects focus on dry collections (e.g., pinned insects, herbaria, mollusk shells or microscope slides). The majority of specimens in crustacean collections, such as in the Museum für Naturkunde Berlin (MfN), are stored in ethanol (wet collection) and are therefore not as easy to digitize as dry specimens or plane microscopic slides. Imaging ethanol-preserved specimens can be time-consuming as they have to be removed from their jars or vials. Before imaging, single specimens either have to be entirely submerged in new fluid to create a single focal plane for imaging (Vollmar et al., 2010) or one has to wait until the ethanol is completely evaporated from the specimen's surface.

The use of digital images of specimens and drawings for taxonomic or other publications in smaller crustaceans instead of using drawings made with a camera lucida are increasing but not standard procedure yet. Digital line drawings of scanned pencil drawings (or photos) using a digitizer board and graphic design software were already successfully applied for scientific illustrations of crustacean morphology (e.g., Coleman, 2003, 2006, 2018; Rodcharoen et al., 2016).

The aim of this student project was i) to test a digitization workflow for a possibly faster description and documentation of morphological characters for species descriptions in isopod crustaceans, ii) to do a small test run for the digitization of ethanol-preserved museum specimens (wet collection), and iii) to test the use of museum internal Uniform Resource Identifiers (MfN URIs or NURIs) and QR code labels to easily connect a physical research object with a database.

2. Material

This student project tested a digitization workflow with ethanol-preserved isopod crustaceans (freshwater and terrestrial from Southeast Asia; Table 1). These isopods were chosen as test organisms as they were recently collected (Table 1) and not preserved in ethanol too long. These are easy to handle due to their robust exoskeleton and their optimal size for stereo microscopy which is usually used for morphological studies and species descriptions of smaller crustaceans of less than 5 cm.

Specimens of MfN's crustacean wet collection are usually organized in lots, i.e., many individuals of the same species from the same collecting event (Vollmar et al., 2010). For collection management, new species lots are inventoried with the institution's acronym 'ZMB', the specific collection term 'Crust.' and a unique identification number (e.g., ZMB Crust. 29000; specimens from Table 1 have not been fully inventoried yet). The species lot number, the taxon name and additional information are then recorded in a collection management database and ethanol-proof labels are placed inside each lot container filled with 75-80% ethanol for long-term preservation of specimens.

As the majority of species examined are not described yet, not all results from Table 1 are shown in this protocol but will be kept for later taxonomic descriptions with isopod specialists (for example, with an Indonesian specialist for the description of the new *Cirolana* species).

Table 1. List of museum specimens of freshwater and terrestrial isopods from this study.

ZMB Lot #*	NURI code**	Family	Species	Year of collection	Locality
**Freshwater isopods from Sulawesi, Indonesia (examined by JLU)**
#	coll.mfn-berlin.de_u_eddde6	Cirolanidae	*Cirolana* sp.	2015	Lake Poso, E shore, S of Tentena, dive at small cape
#	coll.mfn-berlin.de_u_6aeef8	Cirolanidae	*Cirolana* sp.	2018	Lake Poso, W shore, S corner of Siuri bay
#	coll.mfn-berlin.de_u_2555cb	Cirolanidae	*Cirolana* sp.	2015	Lake Poso, E shore, beach in front of Dolidi Ndano Cottages
#	coll.mfn-berlin.de_u_4cd63d	Cirolanidae	*Cirolana* sp.	2018	River at road Malino - Watambayoli
#	coll.mfn-berlin.de_u_44f8a0	Cirolanidae	*Cirolana* sp.	2019	Lake Towuti, W shore, N shore of outlet bay, small cape
#	coll.mfn-berlin.de_u_6e16da	Cirolanidae	*Cirolana* sp.	2017	Lake Poso, E shore, beach in front of Dolidi Ndano Cottages
#	coll.mfn-berlin.de_u_453e1a	Cirolanidae	*Cirolana* sp.	2018	Lake Poso, NW shore, Cape Woku
#	coll.mfn-berlin.de_u_d87170	Cirolanidae	*Cirolana* sp.	2012	Lawa River, c. 50-100 m from Matano Lake
#	coll.mfn-berlin.de_u_4ff1c8	Cirolanidae	*Cirolana* sp.	2012	Lake Towuti, Tanjung (cape) Karipinang, around mouth of Larona River
#	coll.mfn-berlin.de_u_d10439	Cirolanidae	*Cirolana* sp.	2019	Lake Towuti, Loeha Island, SW shore
#	coll.mfn-berlin.de_u_d7a509	Cirolanidae	*Cirolana* sp.	2018	Lake Poso, W shore, Bay N of Bancea
#	coll.mfn-berlin.de_u_534f94	Cirolanidae	*Cirolana* sp.	2019	Matabundu waterfall, Wasuponda (Leduledu)
**Terrestrial isopods (examined by CMAR)**
#	coll.mfn-berlin.de_u_9c4498	Armadillidae	*Armadillidium* sp.	2020	Malaysia: Perak: Ipoh
#	coll.mfn-berlin.de_u_195b81	Armadillidae	*Cubaris murina*	2020	Thailand: Prachuap Khiri Khan: Sam Roy Yot
#	coll.mfn-berlin.de_u_4a69e2	Armadillidae	*Cubaris* sp.	2020	Thailand: Songkhla: Ban Rattabum, Boripat waterfall
#	coll.mfn-berlin.de_u_8521db	Armadillidae	*Cubaris* sp.	2020	Thailand: Prachuap Khiri Khan: Sam Roy Yot
#	coll.mfn-berlin.de_u_4459a0	Armadillidae	*Cubaris* sp.	2020	Thailand: Surat Thani: Amber Tham Sinkorn
#	coll.mfn-berlin.de_u_49cc2e	Armadillidae	*Merulanella bicolorata*	2020	Thailand: Tak: Amber Muang Trang, Taksin N.P.
#	coll.mfn-berlin.de_u_fa9273	Armadillidae	*Merulanella* sp.	2020	Thailand: Nakhon Si Thammarat: Khao Luang Pomkiri
#	coll.mfn-berlin.de_u_76eecc	Armadillidae	*Venezillo* sp.	2020	Thailand: Nakhon Si Thammarat: Lan Saka, Cave Khun Thale
#	coll.mfn-berlin.de_u_be925d	Portellionidae	*Portellio* sp.	2020	Vietnam: Trading, probably near Trak

*ZMB numbers are still pending and were just used for internal use (as species descriptions are expected from this material) and therefore are not listed here.

**NURI codes are just preliminary and are only accessible with an internal MfN Access database (test QR codes are not shown here for the same reason).
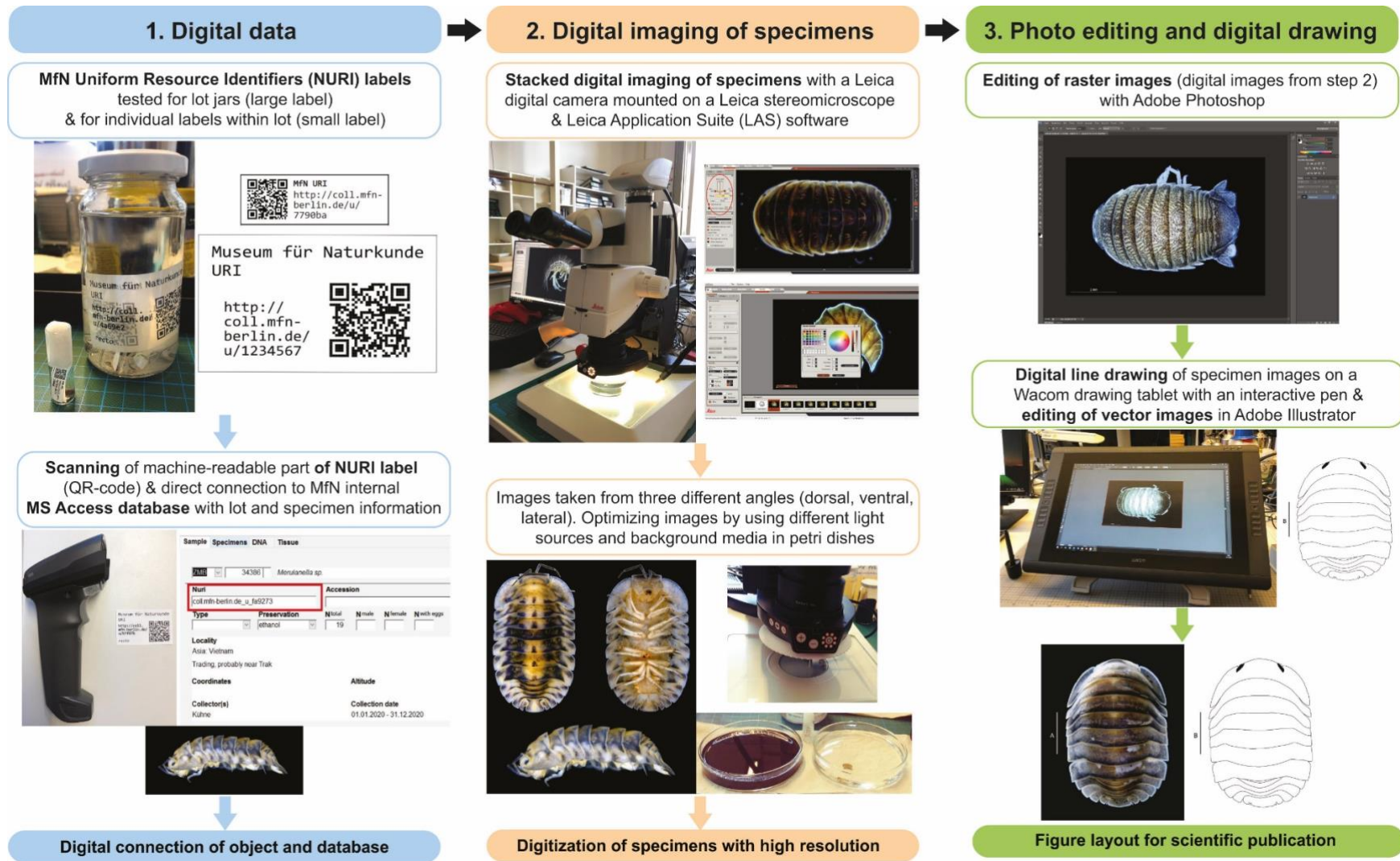
3. Workflow overview

Figure 1. Overview of digitization workflow for ethanol-preserved isopod specimens.

4. Workflow protocol in detail

Part 1. Digital data

Before the project started, all isopod samples from Table 1 were already presorted into lot jars and had preliminary identification and temporary ZMB labels (Fig. 2A). Each lot information (temporary ZMB number, taxon name, collecting event) was already available in a working group internal MS Access database that has previously been created for research-related sample management and could then be used for the test run with isopods. The next step was to separate all individuals from each lot by putting them into small glass vials that were then closed with non-sterile medical cotton balls (Fig. 2B).
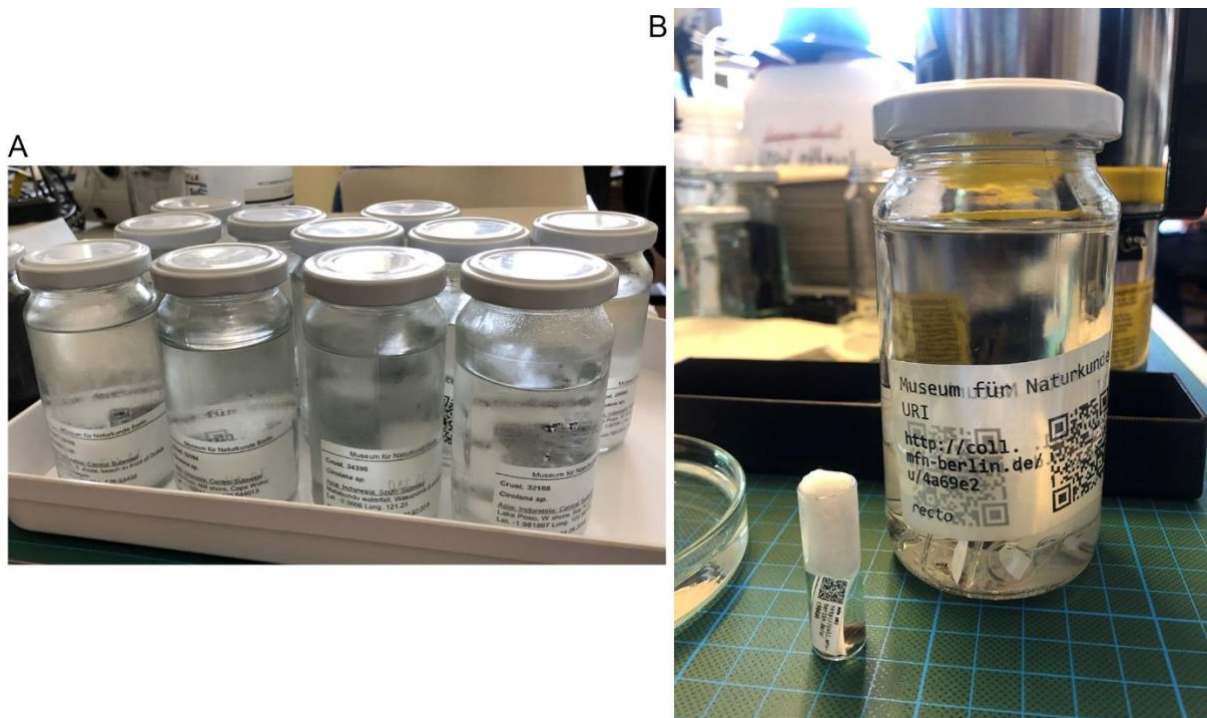

Figure 2. A. Example of presorted species lot jars with preliminary ZMB labels. B. Example of lot jar and individual vial with new NURI labels.

In addition to the ZMB label as standard part of collection management, each lot jar and individual vial was then provided with a MfN internal 'NURI' label (abbreviation for Museum für **N**aturkunde Berlin - **U**niform **R**esource **I**dentifier or MfN URI; Fig. 2B).

A NURI consists of an MfN specific code followed by an individual code, for example http://coll.mfn-berlin.de/u/7790ba or http://coll.mfn-berlin.de/u/1234567 (Fig. 3). NURIs can connect a physical (museum) object with an internet address such as a landing page for a collection object or a database with object information. NURI labels are printed with the NURI text and a machine-readable QR code as shown in Figure 3.

NURI codes and NURI labels were provided by MfN's digitization & data management teams and were printed for this project in two different sizes (Figs. 2B, 3) suitable for individual vials (18 x 6 mm) and lot jars (64 x 40 mm). Both were chosen based on the standard size of ZMB labels, lot glasses and vials used in the MfN crustacean collection. Afterwards, both label sizes were printed on DIN4 label paper that had to be ironed to fix the ink before labels could be cut out by hand (small labels) or with a manual cutting machine (large labels). Ironing of label paper is a standard method of wet collections to make laser printed labels ethanol-proof.

Figure 3. Examples of small and large NURI labels (not to scale) with NURI text and QR-code.

The machine-readable QR code could then be scanned with a QR code scanner (Fig. 4) or a mobile phone camera if the size of the QR codes was not too small. For this test run, the scanner was connected to a desktop computer that had access to the working group's internal MS Access database with already existing isopod lot information (Figs. 5-7).

Figure 4. A NURI label for an isopod lot with a machine-readable QR code and a hand scanner.

The MS Access database was mainly used for research projects to manage and search for samples based on taxon name, ZMB or other museum numbers, lab code, etc. (Fig. 5), to provide lot information (Fig. 6) and specimen details (Fig. 7). The scanned QR code could then directly be imported as a NURI text into the database into an already existing but empty NURI field (see red circle or box in Figs. 5-7) and thus creating an easy and fast connection between isopod object and database information.
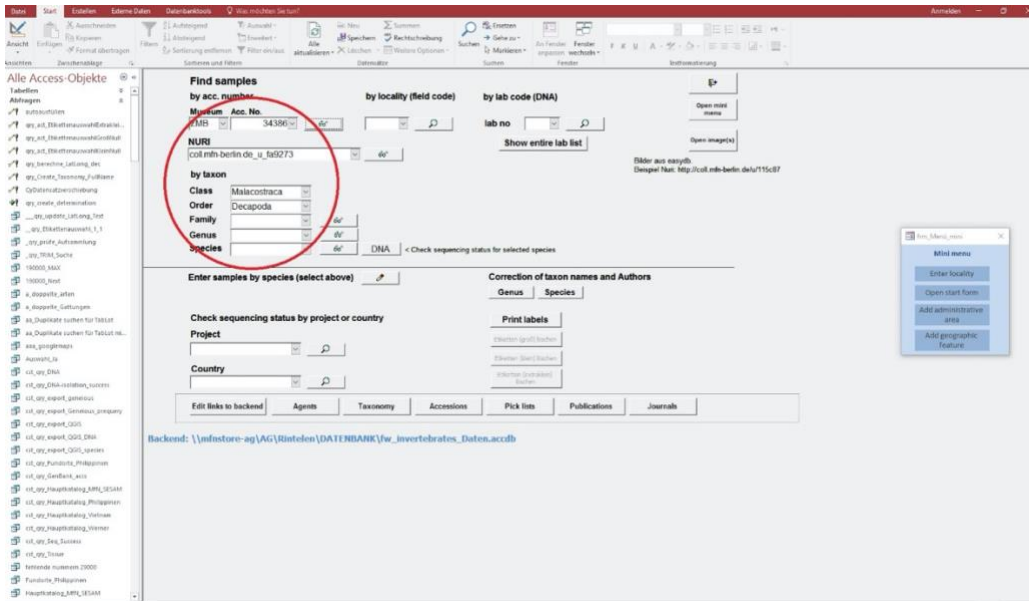
Figure 5. The frontend of the MS Access database with a scanned and imported NURI code.
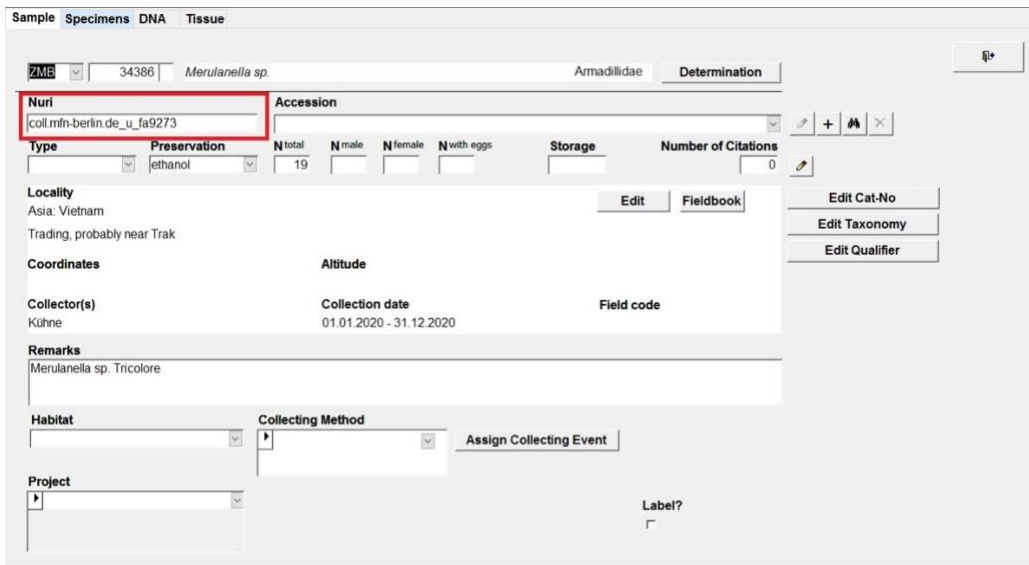

Figure 6. NURI codes can directly be scanned to connect a physical isopod lot with the digital lot information in the database.
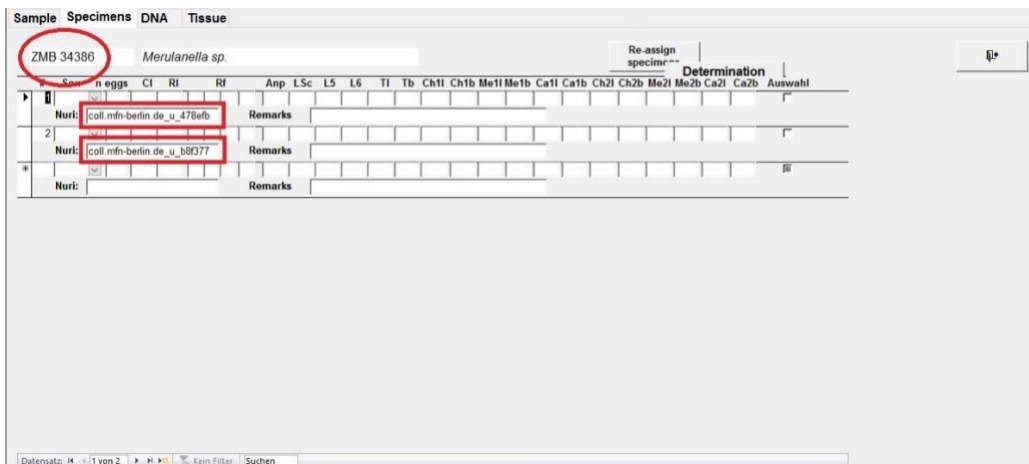

Figure 7. NURI codes can directly be scanned to connect a physical isopod specimen with the digital specimen information in the database.

Results and troubleshooting from part 1 of the workflow (compare Fig. 1)

In general, the use of additional NURI labels to easily connect physical research objects (isopod lots or specimens) with the Access database worked well. This step made it easy to access object information when a NURI label was present. However, there were some obstacles and necessary adjustments:

- In preparation of the project, it was originally planned to use NURI labels printed on both sides (recto and verso; an example is shown in Fig. 8) and non-mirror inverted in case a code on one side gets damaged (e.g., by scratching with forceps). After printing, it was noted that the codes on each side did not overlap exactly (Fig. 8), and especially small labels could not be cut out properly. Due to the limited duration of the project, it was decided to use single-sided NURI labels (Fig. 3) only.

Figure 8. Example of a NURI label printed on both sides, non-mirror inverted.

- The original idea to save time was to put the NURI labels inside the lot jar or specimen vial and scan them from the outside without taking the labels out again. The small NURI labels used for the individual vials (Figs. 2B, 3), were often challenging to scan. All attempts to scan them through the wall of the ethanol-filled glass vial failed. It was necessary to take the labels out again and in some cases to let them completely dry before the scanning process was successful. But even then it took several attempts to scan the code.
- The larger NURI labels (Fig. 3) caused fewer scanning problems. The QR code scanner scanned the code through the wall of the ethanol-filled jar (Fig. 2b) without any problem. However, one precondition was that the label was placed against or very close to the wall of the jar (the large label in Fig. 2B) and was not tilted backwards like, for example, the small label in Fig. 2b.
- The duration of this student project was not long enough to test how long NURI labels in ethanol remain machine-readable (longer than several months or years?). However, some labels were not or hardly scannable after a few weeks and months in ethanol.
- Each database has specific problems and can cause delays. A database manager was needed to add empty NURI field to the respective frontend level (presentation layer; Figs. 5-7) and to generally maintain the database backend of the MS Access database.
- In conclusion, part 1 of the workflow (Fig. 1) can only work if the different hard- and software parts and label options are installed and previously fully tested, respectively. Additional support needs to be considered (IT, database manager, and technical support). Only fully functional, this part of the workflow helps to quickly scan and find object information in the database.

Part 2. Digital imaging of specimens

For the second part of the workflow (Fig. 1) digital images of specimens were generally taken under a stereomicroscope equipped with a digital camera and processed through additional stacking software on a connected computer (Fig. 9). The aim was to produce stacked images that are ideally sharp throughout and have a high resolution (Figs. 12-13).

Figure 9. Workflow equipment for digital imaging of specimens.

For this test run, digital images of single specimens were taken with a Leica DMC6200 camera mounted on a Leica M205 C stereomicroscope. Multiple images were stacked through Leica Application Suite software (LAS v. 4.13; Figs. 20-21) that merged several images together to a TIF file with high resolution. The single photos and stacked TIF images could then be safely stored on an MfN internal server (as 'raw data' from LAS software) and otherwise processed for the third part of the workflow (photo editing and digital drawing; Fig. 1).

Preparation of specimens for digital imaging

Specimens were taken out of their vials and placed in a petri dish either filled with 75% ethanol or with black sand and ethanol (Fig. 10). Black sand helped to stabilize the isopods in a certain position but was not always necessary. Ethanol was always used as specimens fully covered with ethanol caused fewer light reflections on their surface. The alternative, to let the ethanol evaporate first and take pictures of dry specimens either on dry black sand or felt (Fig. 11), was quickly abandoned because it was planned to take DNA tissue samples afterwards that had ideally not fallen dry before. In addition, felt was not ethanol-proof and less flexible than sand.

Figure 10. A petri dish filled with 75% ethanol and black sand (left) and with 75% ethanol only (right).
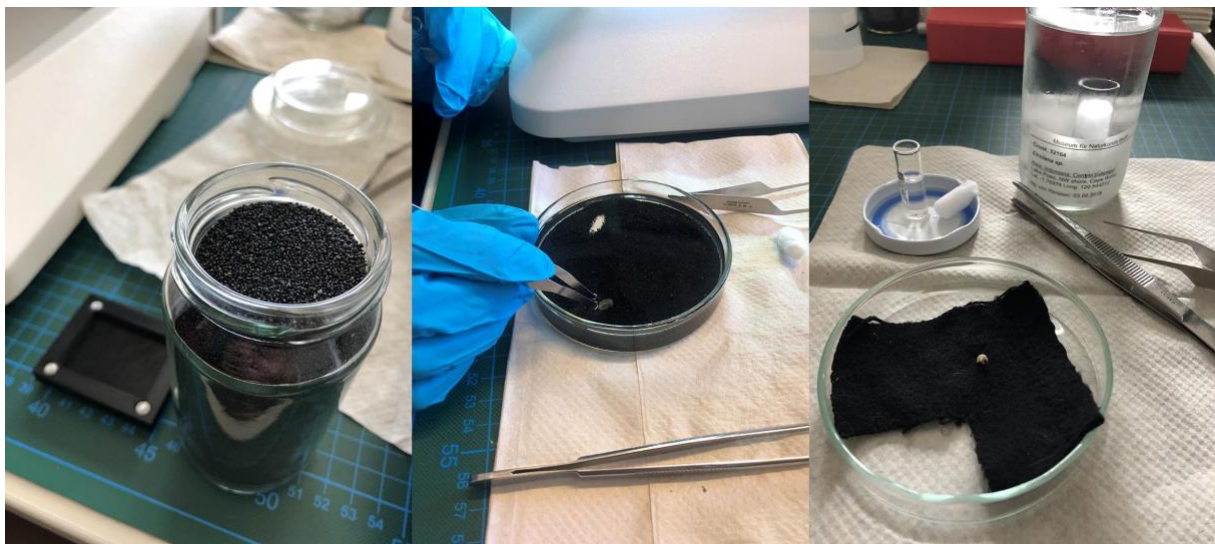

Figure 11. Alternative backgrounds such as dry black sand (left) or dry felt (right) were quickly abandoned as they turned out to be less efficient than black sand with ethanol (middle) or just ethanol.

After choosing the ideal background, images of isopods were generally taken from three different angles: dorsal, ventral, and lateral (Figs. 12-13). In cases when specimens were curled up (Fig. 12) segments and extremities were not fully visible compared to images taken from stretched individuals (Fig. 13). For this test run it was not possible to carefully stretch them.

Figure 12. Curled up individual of *Armadillidium* sp. from Thailand (Table 1) in A. dorsal, B. ventral, C. lateral view. Scale bar A-C: 2 mm.

Figure 13. Stretched individual of *Cubaris* sp. from Malaysia (Table 1) in A. dorsal, B. ventral, C. lateral view. Scale bar A-C: 2 mm.

Light sources

Two different types of light sources were tested: A fixed ring light (Fig. 14) and a flexible cold light source (Fig. 15, red circle). The use of a small plastic dome (Fig. 16) was also tested to reduce light reflection but was not efficient enough. Both light sources were used separately or simultaneously depending on the surface details of the specimen (Figs. 17-19).

Figure 14. A Leica LED3000 compact LED ring illuminator was used in cases when a bright and homogenous illumination of specimens was needed (also visible in Fig.15).
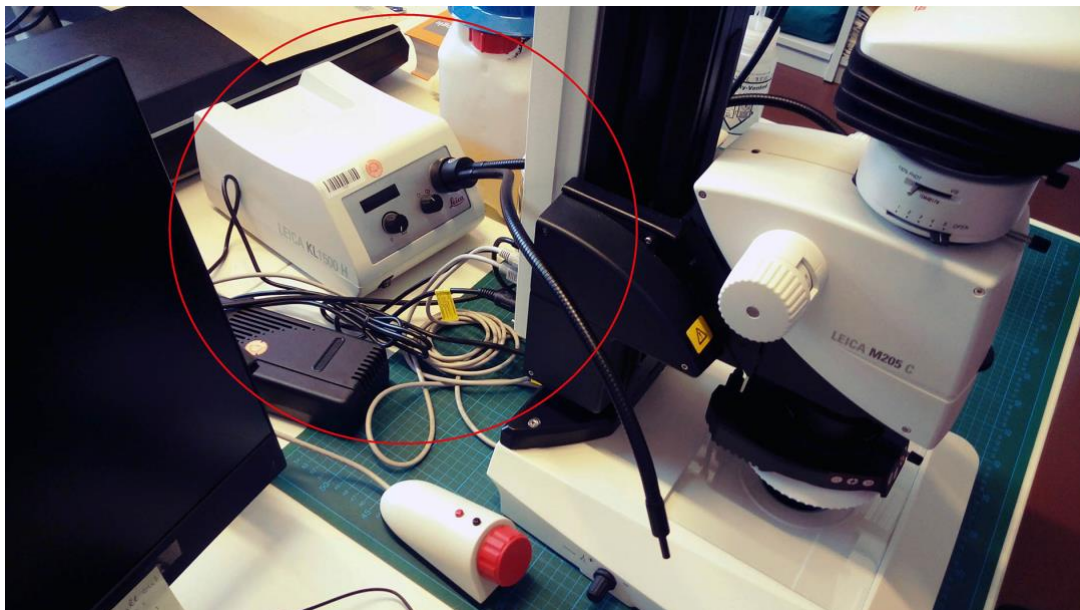
Figure 15. For precise illumination of morphological details, a flexible cold light source (KL 1500 HAL, 150 W, double swan neck light; red circle) was used, sometimes in addition (as shown here), sometimes as alternative to a ring illuminator which was then removed.

Figure 16. The use of a small plastic dome around the sample to reduce light reflection was not efficient enough.

2 mm

Figure 17. Lateral view of *Merulanella bicolorata* (Table 1). Original picture taken with a cold light source only (Fig. 15, red circle).

Figure 18. Lateral view of *Merulanella bicolorata* (Table 1). Original picture taken with a ring light and a cold light source simultaneously (as shown in Fig. 15).

Figure 19. Lateral view of *Merulanella bicolorata* (Table 1). Original picture taken with a ring light only (Fig. 14).

Results and troubleshooting from part 2 of the workflow (compare Fig. 1)

In general, the method and equipment used produced very sharp and high-resolution results.

- Before microscopic pictures of ethanol-preserved specimens can be taken, it is essential to make a test run with different backgrounds (depending on: color, size, and fragility of specimens; the different positions of specimens while taking pictures; dry or wet substrate) and different light sources. Additionally, the software settings (white balance etc.) can also have an effect on color differences as shown in Figs. 17-19 and should be checked as well.
- LAS software in general was often error-prone and did not always run smoothly. For example, stacking with LAS software (Fig. 20) not always produced the best results compared to alternative stacking programs such as Helicon Focus and the LAS interface could have been more user-friendly (Fig. 21).

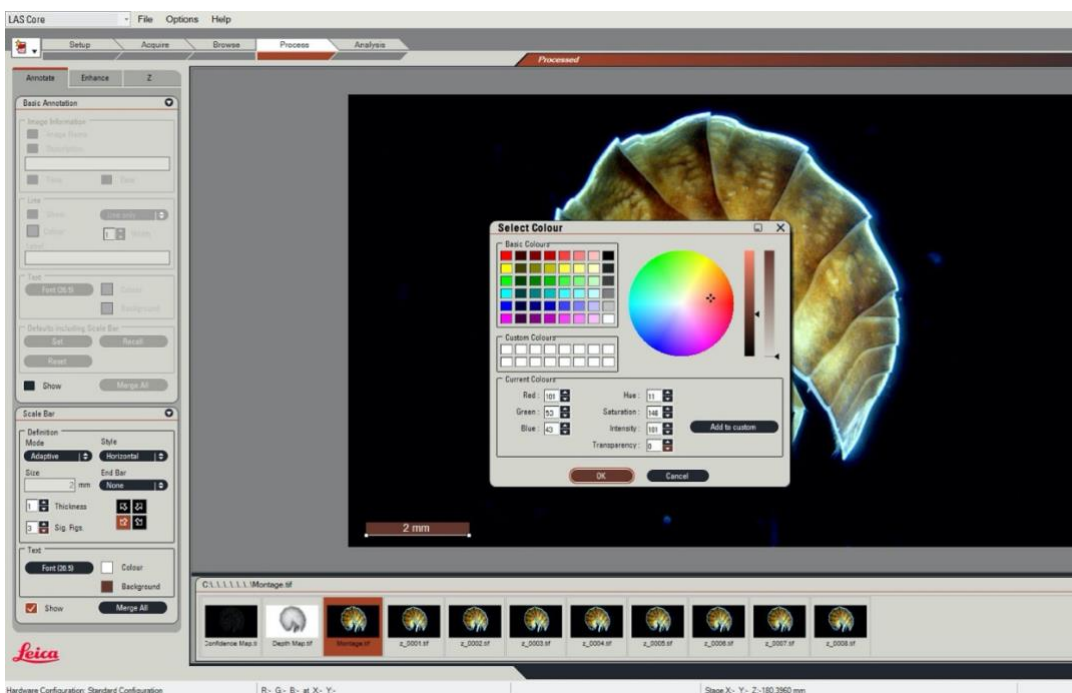**Figure 20.** Stacking settings in LAS (stacking examples are shown in Fig. 21).

Figure 21. Example of LAS interface: editing a scale bar to a stacked image.

Part 3. Photo editing and digital drawing

The stacked TIF images could then be processed for part 3 of the workflow (photo editing and digital drawing; Fig. 1) to produce illustrations for scientific publication as a final result of the workflow.

All illustrations presented here were made with Adobe Photoshop CS6 and Adobe Illustrator CS6 if line drawings were needed. Digital drawings were made with a Wacom Tablet Cintiq 22HD and an interactive pen using Adobe Illustrator similar to Coleman (2003). Final plates with original pixel and, in some cases, also vector images were made in Adobe Photoshop.

Editing of raster images with Adobe Photoshop

The high resolution TIF images were opened in Adobe Photoshop (Fig. 22) and reduced to optimal image size and resolution. Slight adjustments of contrast, brightness, and color were made (Fig. 23). The background was deleted, scale bars redrawn and specimens cropped (Fig. 22).

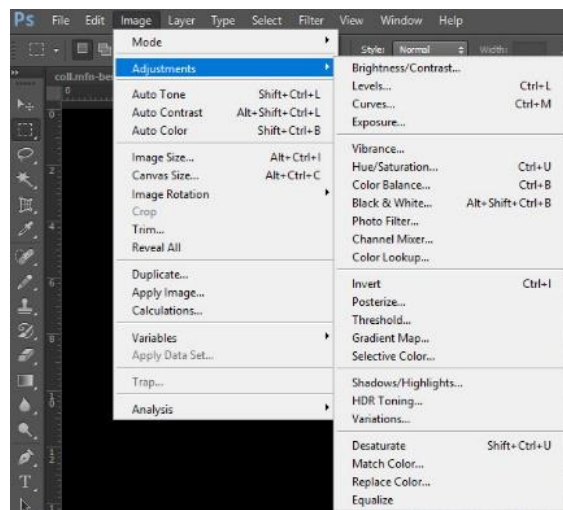**Figure 22.** A stacked image opened in Adobe Photoshop CS6 (specimen already cropped).

Figure 23. Slightly improving image quality with adjustment functions in Adobe Photoshop.

Adobe Illustrator

After photo editing with Adobe Photoshop, images were opened in Adobe Illustrator. Line drawings of specimens and scale bars were made with a Wacom Tablet Cintiq 22HD and an interactive grip pen (Fig. 24). These vector images were edited with Adobe Illustrator (Fig. 25) and the original specimen images deleted afterwards.
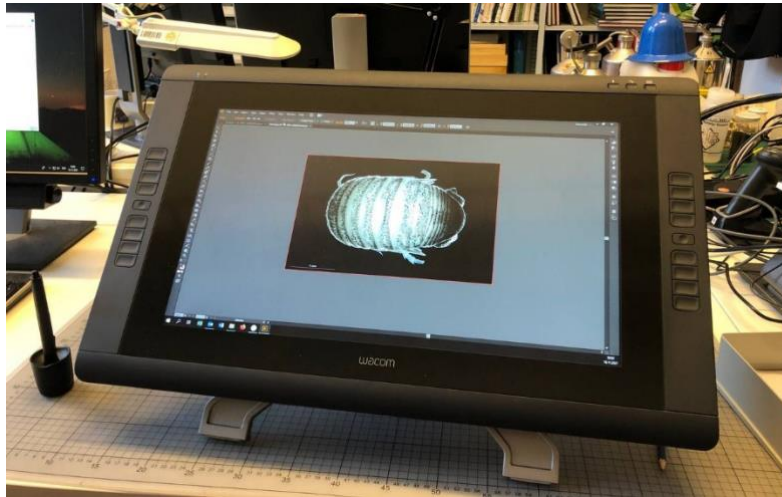

Figure 24. Wacom Tablet Cintiq 22HD and an interactive grip pen next to it.
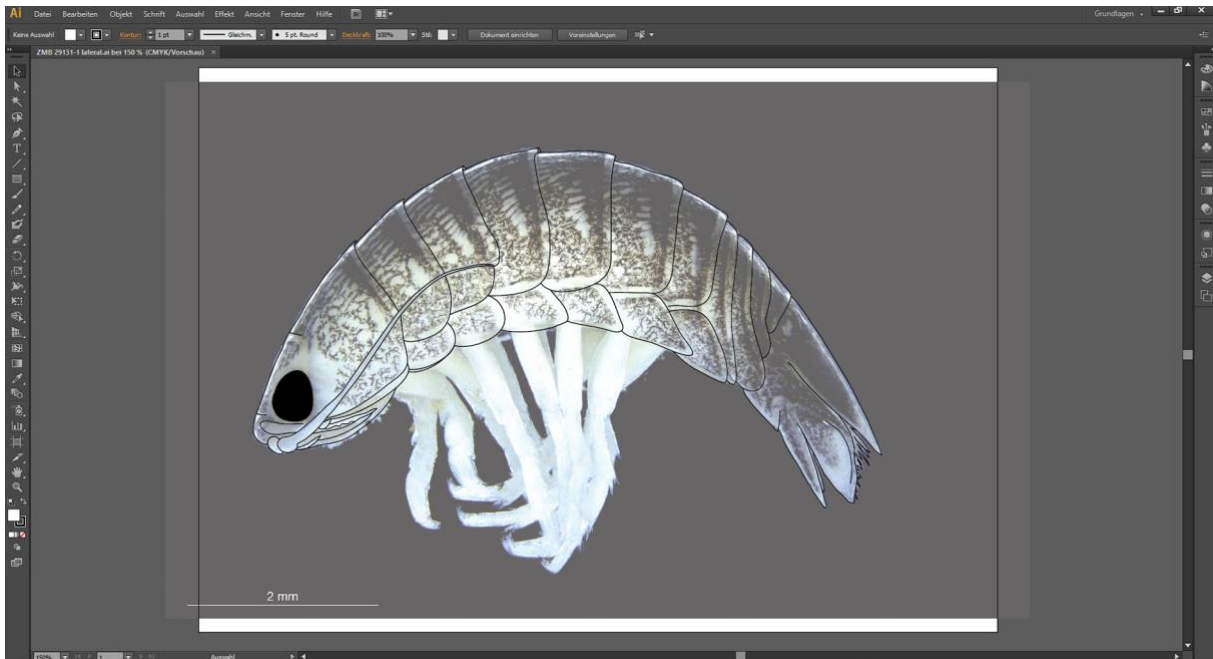

Figure 25. Digital line drawing from an isopod specimen image that was deleted afterwards.

Results and troubleshooting from part 3 of the workflow (compare Fig. 1)

For scientific publications, illustrations are often presented as plates. The final results of part 3 of the workflow (Fig. 1) are shown in Chapter 5. The original illustrations with high resolutions are ready to be used for publication; this workflow shows reduced JPG images.

This protocol is not meant to be used as an overall guide for photo imaging for scientific plates, there are better and more detailed alternatives (such as Bevilaqua, 2020) or as a manual for Leica equipment and software. But one point to consider is the temptation of too much image manipulation with imaging software (see discussion in Rossner & Yamada, 2004). To avoid this, we suggest a rather minimalistic approach and the use of a published protocol (e.g., Bevilaqua, 2020) to make image manipulation as transparent as possible and compatible with good scientific practice before publishing results from this workflow. We also advise to look at journal guidelines about which types of digital manipulation are acceptable as suggested by Rossner and Yamada (2004). As a standard procedure all raw data (in this case, TIF format images in high resolution before photo imaging) from this workflow is stored on the MfN's internal server.

5. Final workflow results – some examples of scientific illustrations

Examples of final workflow results (compare Fig. 1) and plates arranged with Adobe Photoshop and/or Illustrator are shown (Figs. 26-30; see also final plates in Figs. 12-13), either with habitus photographs only (Figs. 12-13, 26-28) or with a combination of photographs and drawings (Figs. 29-30); other combinations or the reuse of single pixel or vector graphics are also possible (not shown here). These are ready to be used for scientific publications of species descriptions or other research results related to the specific taxa. Not all results are shown in this study (compare Table 1) but will be provided for future taxonomic description in collaboration with isopod specialists.

Figure 26. The terrestrial isopod species *Cubaris murina* from Thailand (Table 1). A. dorsal, B. ventral, C. lateral view. Scale bar A-C: 2 mm.

Figure 27. The terrestrial isopod species *Merulanella* sp. from Vietnam (Table 1). A. dorsal, B. ventral, C. lateral view. Scale bar A-C: 2 mm.

Figure 28. The terrestrial isopod species *Portellio* sp. from Thailand (Table 1). A. dorsal, B. ventral, C. lateral view. Scale bar A-C: 2 mm.
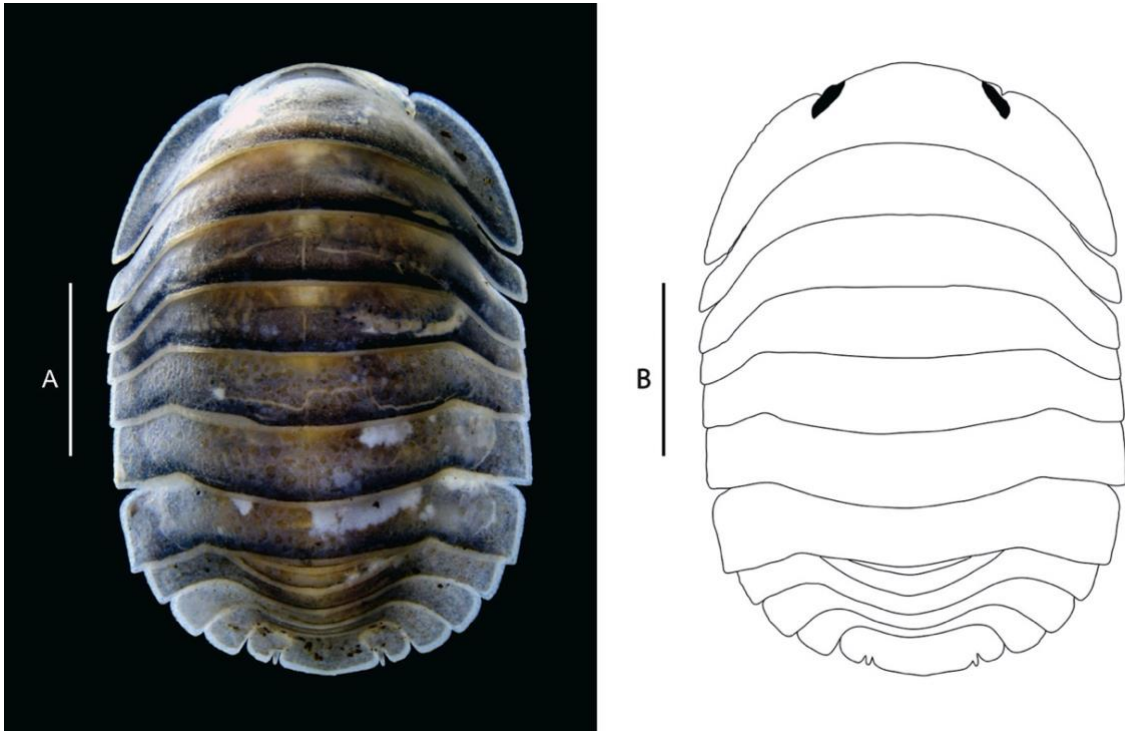
Figure 29. The terrestrial isopod species *Cubaris* sp. from Thailand (Table 1). A. dorsal view, B. line drawing based on picture taken for A. Scale bar: 2mm.
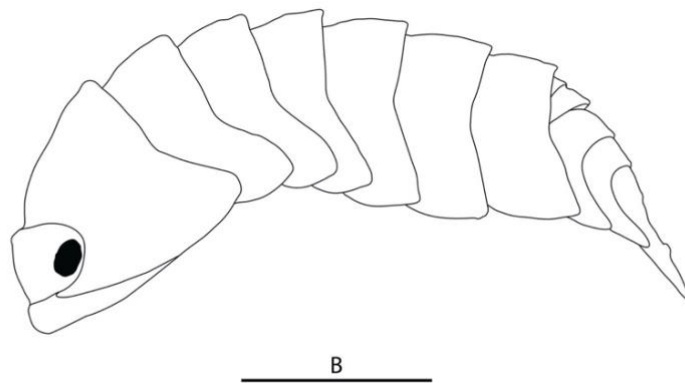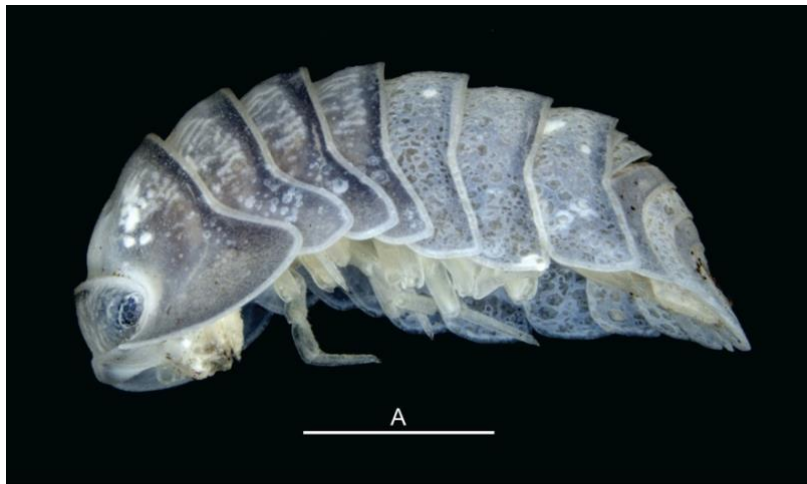

Figure 30. The terrestrial isopod species *Cubaris* sp. from Thailand (Table 1). A. lateral view, B. line drawing based on picture taken for A. Scale bar: 2mm.

6. General discussion

Does the workflow help to accelerate the description and documentation of morphological characters in isopod crustaceans?

Using a standard protocol for microscopic imaging and digital drawing of morphological characters in small isopods and possibly other small crustaceans could indeed help to accelerate the process of species description, which still is a rather time-consuming part of the process. It could become a standard procedure of an integrative taxonomic approach; whereas the use of NURI labels (part 1 of the workflow; Fig. 1) should not be included (see discussion below).

Another advantage is the possibility to share high-resolution images among scientists and others for a discussion of morphological characters or identification issues, for citizen science, conservation projects, or taxonomists interested in these taxa without the possibility of visiting the museum collection. This study just tested habitus images from different angles. We did not test the workflow for an entire species description yet, which usually includes more details and knowledge of appendages or mouthparts, but it has already been successfully tested with freshwater shrimps of the family Atyidae by the third and fourth authors.

Was the test run for the digitization of ethanol-preserved museum specimens successful?

The overall test run was successful; the final images of specimens (after image editing) were of high quality. The workflow may not be suitable for mass-digitization of ethanol-preserved museum specimens as too many steps and different settings are involved.

Was the use of museum internal Uniform Resource Identifiers (MfN URIs or NURIs) & QR code labels helpful in this workflow?

NURI labels helped to track lots or individuals or to quickly lookup collection data for an associated object in the Access database. However, ethanol-preserved QR code labels were not always easy to handle and readable by the scanner depending on the size of the label and their position within the jar; small labels within individual vials did not work at all but had to be taken out and dried before they could be scanned, which was not time-efficient. Whether NURIs and QR code labels will be useful and long-lasting in ethanol-preserved museum collections could not be fully tested due to the limited time of this project (some labels were not or hardly scannable after a few weeks or months in ethanol).

Overall, NURI labels and especially the QR codes had some shortcomings in ethanol, and their use did not lead to the hoped for time-saving efficiency.

Conclusion

The workflow itself proved to be efficient and time-saving but only provided that the entire equipment and software is fully functioning. It may not be suitable for the mass-digitization of wet collections in natural history museums but can help standardize and digitize the morphological part of a taxonomic species description. Furthermore, it can help exchange high-resolution digital images and digital drawings of habitus and taxonomic details of ethanol-preserved museum specimens below 5 cm with other taxonomists or digital images with a broader public.

7. Author contributions

JLU and CMAR equally contributed to this study as part of their individual student projects at the Museum für Naturkunde Berlin. Both helped to develop and test the entire workflow, wrote the protocol and contributed with their own ideas to improve details of the workflow based on their taxa: JLU tested the workflow for yet undescribed freshwater isopod species from the Indonesian island Sulawesi. CMAR tested the workflow with terrestrial isopods from Thailand. VdM gave valuable technical support for the development of the digitization workflow. KvR supervised both student projects and conceived the original idea for this study. VdM and KvR provided critical feedback to the protocol. All authors read and approved the final manuscript.

8. Acknowledgements

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